Title: Rock pool gobies change their body pattern in response to background features

Abbreviated title: Pattern change in rock pool fish

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ABSTRACT

Some species actively change colour and pattern for camouflage on a range of background types. Such dynamic camouflage may be particularly advantageous for species inhabiting heterogeneous habitats, such as intertidal zones, where individuals are exposed to both terrestrial and marine predators depending on tides and wave action. Most studies of dynamic pattern camouflage have focused on relatively few species, and rarely species inhabiting the intertidal zone. We used image analysis and predator (avian) vision modelling to determine if rock gobies (*Gobius paganellus*) change their body pattern in response to their background, and to explore how background marking size influence pattern change. Rock gobies rapidly (within 1 min) changed their pattern when placed on checkerboards with different sized squares, and on backgrounds resembling natural substrates. On backgrounds resembling natural substrates, those with a small grain size, such as sand, elicited a larger degree of pattern change than those with a larger grain size. However, despite this, the majority of fish showed little or no improvement in background matching over time. Instead, the markings elicited are characteristic of disruptive coloration, and may function primarily through breaking up the body outline rather than via improved match to the background pattern itself.

Key words: background matching-camouflage-colour change-disruptive coloration-fish-pattern change.

INTRODUCTION

Many animals use camouflage to conceal themselves from potential predators, and there are countless camouflage strategies in nature (Thayer, 1909; Cott, 1940; Stevens & Merilaita, 2009a). Those that primarily prevent detection by an observer are collectively referred to as crypsis. Arguably the most common of these strategies is background matching, which involves the animal's body resembling the colour, intensity, pattern, and sometimes movement, of one or several background types (Stevens & Merilaita, 2009a). Most natural habitats are not uniform, but comprise considerable spatial and temporal variation. This variation creates a problem for effective camouflage because concealment depends on an interaction between the animal and the background. To overcome this problem, some species exhibit behavioural preferences for backgrounds that match their coloration closely, either at a species or individual level (Kettlewell & Conn, 1977; Stoner & Titgen, 2003; Hultgren & Stachowicz, 2011; Kang *et al.*, 2012, 2013; Lovell *et al.*, 2013; Marshall, Philpot, & Stevens, 2016).

An alternative, or additional mechanism to behavioural choice in coping with heterogeneous habitats is the ability to change colour in response to the background (Stuart-Fox & Moussalli, 2009; Stevens, 2016). For example, cuttlefish are able to rapidly (within seconds) change their body pattern in response to changes in the size, colour, and composition of their visual background (Mäthger *et al.*, 2007; Barbosa *et al.*, 2008b) and so improve camouflage as perceived by predators (Chiao *et al.*, 2011). Flatfish are also well known for their ability to change body pattern in response to both natural and artificial backgrounds (Sumner, 1911; Fujimoto *et al.*, 1991; Ramachandran *et al.*, 1996; Healey, 1999; Kelman, Tiptus, & Osorio, 2006). Rapid colour and pattern

68 change has also been reported in the slender filefish (*Monacanthus tuckeri*) (Allen *et al.*,
69 2015).

70 Rapid dynamic camouflage is likely to be advantageous in heterogeneous
71 habitats, such as rocky intertidal zones where substrate type can vary substantially and
72 where a range of different background patterns can exist within a very small area.
73 Species inhabiting the intertidal zone are often exposed to many different predators,
74 depending on tidal height, and the action of waves and currents can force animals onto
75 different background types. Common rock pool species from a variety of taxa change
76 colour for camouflage (Keeble & Gamble, 1899; Fries, 1942; Stevens, Lown, &
77 Denton, 2014a; Stevens, Lown, & Wood, 2014b). One such species is the rock goby
78 (*Gobius paganellus* Linnaeus, 1758), which rapidly changes its luminance (perceived
79 brightness) and colour for camouflage (Stevens *et al.*, 2014a). Rapid physiological
80 colour change in this species is likely to be mediated by the movement of pigment
81 organelles within chromatophores (specialised pigment cells). For instance, brightness
82 is controlled by melanophores which contain the pigment melanin (Burton, 2002; Sköld,
83 Aspengren, & Wallin, 2013).

84 The rock goby is an abundant intertidal species around the UK that is exposed to
85 both marine and terrestrial predators depending on tidal height (Stevens *et al.*, 2014a). It
86 therefore makes an ideal species for studying pattern change for camouflage within the
87 intertidal zone. Past work on rock gobies focused on colour and luminance, but not
88 pattern (Stevens *et al.*, 2014a). Observations made in the field suggest that rock gobies
89 are capable of changing their body pattern, however this has never been tested
90 empirically. Quantifying the degree of pattern change and its effect on camouflage

matters here and in other animals because the efficacy of this strategy should have a major bearing on its survival and evolutionary value.

In this study we tested the ability of the rock goby to change its pattern in response to backgrounds with different sized markings. Digital image analysis and a model of predator vision were used to quantify changes in body pattern. The study consisted of two experiments. Experiment 1 aimed to quantify whether rock gobies change their body pattern in response to their background. For this we used checkerboards consisting of different sized black and white squares similar to those used in classic experiments on cuttlefish and flatfish (e.g. Ramachandran *et al*., 1996; Barbosa *et al*., 2008b). Studies on cuttlefish have shown that the size of background markings/objects, relative to the size of the animal, affects their body pattern (Barbosa *et al.*, 2007, 2008b; Kelman *et al.*, 2007; Kelman, Osorio, & Baddeley, 2008). The second experiment therefore aimed to determine if, and how, pattern change in gobies is influenced by the grain size of backgrounds that resembled natural substrates. For this, grey-scale images of different black and white aquarium substrates (sand, gravel and stones), ranging in size from < 1 mm to 40 mm in diameter, were used as the backgrounds on which the fish were tested. Grey-scale images allowed us to test the effect of natural looking backgrounds with different marking sizes while keeping all other information about the background constant (i.e. achromatic, chromatic, and textural information were all controlled). In both experiments we also asked whether pattern change enhanced background matching camouflage.

METHODS

Experimental backgrounds

Backgrounds for both experiments were printed on waterproof paper (Xerox Premium NeverTear) using a Hewlett Packard LaserJet 500 colour M551 PCL6 printer. Experiment 1 followed previous studies on cuttlefish and flatfish that used black and white checkerboard backgrounds to investigate how the animals changed their body pattern in response to different check sizes (e.g. Ramachandran *et al*., 1996; Chiao and Hanlon, 2001; Barbosa *et al.*, 2008b). Two experimental backgrounds were created using black and white squares of two different sizes arranged into a checkerboard (see supplementary Figure S1). The backgrounds were generated in the graphics program inkscape v0.48 whereby a RGB value of 0 was used for the black squares and a value of 255 for the white squares. The checkerboard squares measured either 1 x 1 mm (small check size) or 5 x 5 mm (large check size). Fish used in our experiment ranged in size (measured in standard length, i.e. length from the snout to caudal peduncle) from approximately 90 mm to 40 mm, such that the small checkerboard squares measured between 1.11% and 2.5%, of the standard length of the fish while the large checkerboard squares measured between 5.56% and 12.5%. The rationale for choosing these two checker sizes was somewhat arbitrary but they were subjectively noted to elicit a noticeable change in body pattern in a preliminary study.

To ensure all fish had the same starting point an intermediate grey that matched the mean brightness of the checkerboards was used as a starting background on which all fish were placed before beginning the experiment. This was generated by creating a grid of grey squares starting with a RGB value of 0 (black) and increasing in increments of 2 all the way to values of 255 (white). The grid was then printed and photographed,

followed by measuring the reflectance values of the camera's longwave (LW), mediumwave (MW), and shortwave (SW) image channels (see image analysis below). An Iwasaki eyeColor MT70D E27 6500K arc lamp was used as the light source for these photos. The green channel was used as a measure of brightness in accordance with previous work that used similar techniques to those described here (Spottiswoode & Stevens, 2011; Stevens, Rong, & Todd, 2013). The actual reflectance for the intermediate grey was ~49% since the reflectance of the black and white squares were ~8% and ~90% respectively.

For experiment 2, we used printed grey-scale 8-bit JPEG photographs of black and white aquarium substrates (equal proportions of each) of different sizes (see supplementary Figure S2). These backgrounds were sand (small grain size; < 1 mm in diameter), gravel (medium grain size; 5-8 mm in diameter), stones (large grain size; 20-40 mm in diameter), and a mixture of all three (mixed sizes). In the mixed background, each substrate type of both black and white were approximately evenly distributed. The backgrounds were matched with regards to mean brightness based on image analysis. A detailed description of how these four backgrounds were generated, and where the aquarium substrates were sourced from, is provided alongside supplementary Figure S2. The starting background was created in the same way as in experiment 1, whereby a grey of the same mean brightness as the experimental backgrounds was chosen from a printed grid.

Experimental set up

The experiments were undertaken in a 400 x 300 x 65 mm grey plastic tray. The tray was divided into four separate sections using 3 mm thick acrylic walls that were fixed in

161 place using aquarium safe silicone adhesive. The four sections were in turn split in half
162 by removable 2 mm thick acrylic dividers, each held in place by transparent slide
163 binders that were glued to the walls using the silicone adhesive (see supplementary
164 Figures S1 and S2). The dividers facilitated the movement of fish from one section to
165 another without the need for further handling. Easy movement between sections was
166 important because previous work reported that rock gobies sometimes elicited a
167 darkening of the skin in response to stress during handling (Stevens *et al.*, 2014a). A
168 similar response has also been reported in the goby *Gobius minutus* (Fries, 1942) as
169 well as some species of crustaceans such as the fiddler crab *Uca capricornis* (Detto,
170 Hemmi, & Backwell, 2008). The use of sliding doors to facilitate the movement of fish
171 between the starting and experimental background ensured handling was minimal thus
172 greatly reducing any stress related colour change. Each of the eight compartments
173 measured approximately 85 x 13 mm. In both experiments, the bottom and sides of the
174 four middle compartments were covered with the starting grey, while the four outside
175 compartments were covered with either the small or large check size in experiment 1, or
176 the sand, gravel, stones or mixed grey-scaled backgrounds in experiment 2. The
177 backgrounds were glued to the sides of the tray as well as the bottom as both vertical
178 and horizontal features have been found to influence pattern change in animals such as
179 cuttlefish (Barbosa *et al.*, 2008a; Ulmer *et al.*, 2013). The tray was filled with fresh
180 seawater to a depth of approximately 20 mm. Fresh seawater was used for each fish and
181 the fixed acrylic walls prevented the flow of water between the four sections.
182
183 ## Experimental procedure

184 The experiments were carried out *in situ* on Gyllyngvase beach, Falmouth, Cornwall,
185 UK (50.1441° N, 5.0684° W) between the start of May and end of June 2015. Fish were
186 collected by hand and dip net from rock pools and placed in a grey bucket containing
187 fresh seawater. The rock goby is a very common fish with often several individuals
188 found in the same rock pool, so multiple testing was extremely unlikely, particularly
189 given that they were sampled over a large area. Forty fish were tested in experiment 1
190 and 80 fish in experiment 2 (20 individuals per background). All work was conducted
191 under approval from the University of Exeter Biosciences ethics committee (application
192 2015/739). Gyllyngvase beach is public land and no further licenses or permits were
193 needed. After being tested, all individuals were immediately returned unharmed to their
194 original rock pool area. Rock gobies are not an endangered or protected species.
195 The general protocol was similar to that used by previous research on colour
196 change in this species (Stevens *et al.*, 2014a). Individuals were tested in size matched
197 blocks in which fish were tested simultaneously within 15 min to ensure any differences
198 in pattern change between treatments were not the result of testing fish on different
199 days, or at different times of day. For experiment 1, there were two fish in each block,
200 while for experiment 2 there were four fish in each block. Before starting the
201 experiment, each fish was first placed on the grey starting background and allowed to
202 acclimatize for a minimum of 15 min. This was done to reduce individual differences
203 between fish and to ensure that all fish acclimated to the same background before
204 starting the experiment. This was important because the fish had been collected from
205 different rock pools often consisting of very different substrate types. The
206 acclimatization period also reduced the probability of the results being affected by any
207 stress-induced colour change as result of handling.

Following this, the fish were photographed in both visible and ultraviolet (UV) light and then immediately moved to the experimental background by lifting the removable divider that separated the two sections. Each fish was then photographed at intervals of approximately 1, 5, and 30 min in experiment 1, and at 15 min in experiment 2 (few changes occurred after this time; see results). Although not the primarily interest of this study, it was possible that pattern change could also depend on the size of the fish. To control for this, we therefore measured the standard length (snout to caudal peduncle) of each fish before releasing them and included this in the analysis.

All photographs were taken using a Nikon D7000 digital camera that had undergone a quartz conversion to enable photos to be taken in both visible and UV light (Advanced Camera Services, Norfolk, UK), and fitted with a Nikon 105 mm Nikkor lens. All photos were taken in RAW format with manual white balance and fixed aperture and ISO settings using manual focus. The lens was refocused between the visible and UV photos to maintain the sharpness of each image. The human visible photos were taken using a UV/infrared (IR) blocking filter which transmits wavelengths of 400-700 nm (Baader UV/IR Cut/L Filter). The UV photos were taken using a UV pass and IR blocking filter which transmits wavelengths between 300 and 400 nm (Baader U filter). A custom made filter slider was used to quickly move between the two filters. To account for difference in lighting conditions at different times, and on different days a black and white Spectralon reflectance standard (made from 10 x 10 mm sections of zenith diffuse sintered PTFE sheet, Labsphere), calibrated to reflect 8.3% and 94.7% of all wavelengths respectively, with a scale bar was included in all photos taken. It was important to ensure that the standard was viewed under the same light conditions as the fish. For this purpose, the standard was placed in a custom made

waterproof box which was positioned next to the fish in all photos. The box was made out of clear plastic that allowed both visible and UV light to pass (Sunbed Grade UV Perspex acrylic sheet, Bay Plastics Ltd). A lid was placed over the box between photos to ensure the standard was not contaminated by dust or splashes of water. A tripod was used to position the camera directly above the fish and the standard at a height of approximately 50-70 cm, and a black and silver photographic umbrella (Neewer, Guangdong, China) was used to ensure that lighting was even across the whole tray.

Image analysis

Pattern analysis was conducted using the 'Multispectral Image Calibration and Analysis Toolbox' (Troscianko and Stevens 2015). The visible and UV photos were first combined into a single multispectral image consisting of information from both the visible and UV channels, following linearization of each image and standardization to control for non-linear image responses to light levels and illuminating conditions (Stevens *et al.*, 2007; Troscianko & Stevens, 2015). Following this, a 20 mm scale bar was added and the area of the fish's body (not including the gills, eyes, or pectoral and caudal fins) was selected by hand and saved as a 'region of interest' (ROI).

In accordance with previous work, changes in pattern and camouflage were analysed with regards to the visual system of shorebirds, which are likely to be a key predator of rock pool fish at low tide (Stevens *et al.*, 2014a). The majority of shore birds have a 'violet' sensitive visual system (Ödeen, Håstad, & Alström, 2009). In birds with a violet sensitive system, the sensitivity of the UV cone type is shifted to slightly longer wavelengths than species which have an 'ultraviolet' sensitive visual system, including other potential predators such as gulls (Ödeen *et al.*, 2009). Although gulls differ from

shorebirds in that they have a UV visual system (Ödeen *et al.*, 2009), the differences in the perception between these two systems is likely to be small since both the backgrounds and the fish had relatively low levels of UV reflectance and both UV and many VS birds still see UV light but differ in their sensitivity. We mapped the images to avian vision based on spectral sensitivity data from the peafowl (*Pavo cristatus*) (Hart, 2002), using a mapping technique to convert from camera to avian colour space under a D65 standard irradiance spectrum (Stevens *et al.*, 2007; Troscianko & Stevens, 2015). This mapping is highly accurate in deriving predicted cone catch values compared to methods using reflectance spectra (Stevens & Cuthill, 2006; Pike, 2011; Troscianko & Stevens, 2015). The peafowl is often used as a model species for modelling birds that have a 'violet' sensitive visual system (e.g. Stevens *et al.*, 2014a; Stevens, Lown, & Wood, 2014c; Marshall *et al.*, 2016; Troscianko *et al.*, 2016).

To analyse changes in pattern over time we used a 'granularity' analysis which has previously been used to study camouflage in cuttlefish (e.g. Barbosa *et al*. 2008b; Chiao *et al*. 2009) and shore crabs (Stevens *et al.*, 2014b), and pattern in cuckoo eggs (e.g. Stoddard & Stevens 2010). Granularity analysis is used to analyse the contribution that different marking sizes make to a given pattern (Stoddard & Stevens, 2010). Since the original photographs were taken at slightly different heights, each of the original calibrated multispectral images were standardised to 37 px/mm, before undergoing a fast Fourier transformation and applying 19 octavewide, isotropic band-pass filters to produce 19 images, referred to as 'granularity bands', each containing information at a different spatial scale. These filters function like a sieve starting with small markings (2 pixels) and increasing in size to larger markings (up to 1024 pixels) with a scale incrementing from 2 to the square root of 2 (Stevens *et al.*, 2014b; Troscianko &

Stevens, 2015). Pattern analysis was conducted in Image J using the toolbox's 'Batch Multispectral Image Analysis' tool (Troscianko & Stevens, 2015).

The granularity bands were used to quantify how the overall pattern changed over time once the fish had been exposed to the experimental backgrounds. Overall pattern 'energy' was calculated for each granularity band as the sum of the squared pixel values in each image divided by the number of pixels in the image (Chiao *et al.*, 2009; Stoddard & Stevens, 2010). The greater the pattern energy for any given granularity band the more dominant that particular marking size. The values of energy across all 19 band-pass filtered images produce a 'granularity spectrum' which can be plotted as energy versus pixels (marking size) (Chiao *et al.*, 2009; Stoddard & Stevens, 2010; Stevens *et al.*, 2014b).

Most previous work using granularity spectra has derived descriptive statistics from the spectra for each individual. However, the problem with this is that it can miss considerable information about spectral shape and metrics can be affected by issues such as multi-peaked spectra. In addition, those metrics do not directly compare the granularity of the animal to the background or individual at other time points. We therefore instead adopted a modified recent approach to quantify changes in pattern over time by calculating the sum of the absolute pattern energy difference (PED), at each spatial scale, between two different granularity spectra (i.e. spectra from two different multispectral images) (Troscianko & Stevens, 2015; Troscianko *et al.*, 2016). Here, the granularity spectrum of each fish at the start (0 min) was compared with the spectra of the same individual at each of the other time points (1, 5, and 30 min for experiment 1, and 15 min for experiment 2) to determine changes in fish pattern with time. To quantify changes in background matching over time, the granularity spectrum of each

fish at each time point was compared with the granularity spectrum of the background it was tested on. To obtain the granularity spectra of the backgrounds, each background was photographed in the tray (without water) and analysed in the same way as above. For the ROI the whole area of the background at the bottom of the tray was selected.

Statistical analysis

When analysing the change in pattern over time, the distribution of the pattern energy difference, or PED, values exhibited some positive skew so were natural log transformed prior to analysis to improve the assumption of normality. For change in pattern over time, a PED of zero would be predicted if the fish did not change their body pattern. To test for pattern change as simply as possible we first used a series of one sample t-tests (a separate test for each time point) to determine whether mean PED differed from zero. We did this for time points 1, 5, and 30 min for experiment 1, and for 15 min in experiment 2. Subsequently, to analyse all data from experiment 1 simultaneously we used a linear mixed effect model with PED as the response variable, time point as a fixed effect (1, 5, and 30 min) and fish identity (ID) as a random effect factor. The later accounts for non-independence between time points and prevents pseudoreplication. Note that there is no data point for 0 min because this was used as the reference category against which all other time points were compared. Fish length and background were included as additional fixed effects to see if these influenced PED, and all pairwise interactions of fixed effects were also fitted. For experiment 2 a similar linear model was used, but without time as a fixed effect (because there was only a PED for 15 min), or the random effect since there were no repeat measures. We used model simplification to test for significant interactions and fixed effects.

To analyse difference in pattern between the fish and their background (i.e. background matching) the values for PED did not need transforming as they already fitted a normal distribution. Linear mixed effects models containing background, fish length, and time point as fixed effects, and fish ID as a random effect were used for both experiments. A random effect was required for both experiments because this analysis included a data point at 0 min (i.e. repeat observations on individual fish were present). Interaction terms between fixed effects were initially included as above and model simplification used to sequentially remove non-significant terms (retaining main effects for any variables involved in significant interactions). All analysis and graphing was done in R (R Core Team, 2014) with linear models fitted by maximum likelihood and lme4 (Bates *et al.*, 2014) used for the mixed models. Statistical inference was based on F tests (linear models) and likelihood ratio tests (LRT; mixed models).

RESULTS

Experiment 1: Change in pattern over time

For fish on both backgrounds there was a significant difference in pattern between 0 and 1 min (One Sample t-test: $t=55.83$, $df=39$, $p<0.001$; Figure 1A), 5 min ($t=56.78$, $df=39$, $p<0.001$), and 30 min ($t=67.77$, $df=39$, $p<0.001$). The greatest change in pattern occurred between 0 and 30 min, though it is evident from Figure 1A that the majority of change in pattern occurred within the first minute of being placed on the experimental background. Indeed, analysis using a general linear mixed effects model found no significant difference in the PED (pattern energy difference) across the different time points (discounting 0 min which was used as the reference against which all other time points were compared) (LRT: $\chi^2_{(2)}=4.6$, $p=0.101$). From Figure 1A it also appears that

the small check size background elicited a greater change in body pattern than the large check size background (particularly at 5 min). However, this difference was not statistically significant ($\chi^2_{(1)}$=2.74, p=0.098). There was, however, a significant effect of fish length ($\chi^2_{(1)}$=7.98, p=0.005), whereby larger fish tended to show a greater PED than smaller fish (see supplementary Figure S3A). This trend is most apparent at 5 min. The exception is at 30 min for fish on the large check size, which show no real effect of fish length. There also appears to be more variation in PED between fish within the first 5 min with less variation being observed between fish after they had been on the background for 30 min.

Experiment 1: Change in background matching camouflage over time

The difference in pattern between the fish and their background is shown in Figure 1B. Background matching was better (i.e. the PED between the granularity spectra of the fish and its background was smaller) on the small check size than on the large check size ($\chi^2_{(1)}$=72.54, p<0.001). There was also a significant interaction between time and fish length ($\chi^2_{(3)}$=15.59, p=0.001; supplementary Figure S4A) resulting from an improvement in background matching over time within larger fish (though this is not seen at 30 min for fish on the large check size). However, for fish below ~60 mm in length there was very little or no change in background matching camouflage over time.

Experiment 2: Change in pattern over time

As in experiment 1, there was a significant difference in pattern between 0 min and 15 min (One Sample t-test: t=78.94, df=79, p<0.001; Figure 2A), with the greatest PED being seen within fish tested on the sand background (F-test: $F_{3,75}$=4.05, p=0.01). There

was a similar trend to that seen in response to the checkerboard backgrounds in experiment 1, whereby as fish length increased PED also increased ($F_{1,75}$=14.37, p<0.001; supplementary Figure S3B), although this trend was much weaker in fish tested on the stones background. To test whether this trend was due in part to the influence of a few individuals above 70 mm, the data were remodelled with these data points removed. The same trend was observed, and the effect of background and fish length were both significant when fish over 70 mm were removed from the model. It is worth noting that overall the four substrates elicited less pattern change (a lower PED) than the two checkerboard backgrounds used in experiment 1.

Experiment 2: Change in background matching camouflage over time

The difference in pattern between the fish and their background is shown in Figure 2B. There was a significant three way interaction between background, fish length, and time (LRT: $\chi^2_{(3)}$=13.69, p=0.004). This three way interaction is the result of an effect of length over time for fish placed on the sand and mixed backgrounds, whereby larger fish show a greater improvement in the level of background matching after 15 min, but there was no effect of fish length over time for fish placed on the gravel and stone backgrounds (see supplementary Figure S4B). When the data points for the few fish over 70 mm in length were removed from the model, the three way interaction was no longer significant ($\chi^2_{(2)}$=0.5, p=0.92). This is perhaps not surprising since while excluding fish >70 mm does not reduce sample size substantially, it does reduce the observed range of length by as much as 50 mm (as the body length distribution itself is positively skewed with small number of large individuals). Nonetheless, in this model there remained a significant interaction between fish length and time ($\chi^2_{(1)}$=4.13,

p=0.042), whereby there was a weak negative correlation between fish length and PED. This means that there was an overall improvement in background matching as fish length increased (but this increase was hidden on gravel and stones due to a few overly influential outliers above 70mm). It should, however, be noted that any improvement in background matching was small and may not necessarily effect the detectability of the fish by predators. This is because even after 15 min the PED between the fish and its background was still very high. In addition the difference in background matching between the different backgrounds remained highly significant when fish greater than 70 mm were excluded from the model ($\chi^2_{(3)}$=362.26, p<0.001).

DISCUSSION

We found that rock gobies change their body pattern in response to changes in their visual backgrounds. Overall, in both experiments, pattern change was very rapid with the majority of change occurring within the first minute of being placed on the background. The ability to rapidly change pattern for camouflage when exposed to a new background is likely to reduce the predation risk for animals living in heterogeneous environments. Rapid pattern change may be particularly advantageous for intertidal species such as the rock goby which, when in the open, were observed to be quite active and tended to use bursts of movements followed by several seconds or minutes when they remained still. It was common for the fish to cross, and stop on, many different background types while moving around in this way.

It was also found that larger fish displayed a greater change in pattern than smaller individuals. Interestingly, despite changing pattern when placed on a new background, there was relatively little improvement in the level of background matching

over time for the majority of fish tested. There was, however, an improvement in background matching as fish size (measured as standard length) increased, and observed changes were sometimes dependent on the type of background, perhaps suggesting selection pressure for better background matching increases with fish size. For instance, larger individuals may have fewer hiding places than smaller fish. It may also be easier for predators to spot larger fish in a shallow rock pool. It is, however, important to recognise that larger fish are older and will have been exposed to selection by predators for longer than smaller individuals. As such it may be expected that larger and older fish should, on average, be better at camouflaging themselves than smaller individuals.

Because the backgrounds were all grey-scale and matched in overall brightness (within each experiment), we can be confident that the fish were responding to differences in background pattern and not overall achromatic or chromatic differences. Moreover, the findings show that visual cues alone are sufficient to elicit a change in body pattern. This does not however rule out the possibility that tactile cues may also be important. To our knowledge, our study is one of relatively few undertaken to experimentally investigate the role of specific background features, in this case substrate size, in influencing pattern change for camouflage in fish species other than flatfish. Such tests are important in understanding the adaptive value of colour change for camouflage since the change in camouflage produced should have an effect on survival probability. Future work needs to test how the fish respond to real substrates and also how predators respond to such changes in camouflage produced.

In experiment 2 the change in pattern over time was greatest among fish placed on the fine sand background. It may be that there is a higher selection pressure by predators on backgrounds such as sand that have a small grain size; e.g. there may be

fewer places to hide in comparison to backgrounds with a large grain size where fish may be able to shelter between or under rocks. The smallest change in body pattern was observed in fish tested on the medium (i.e. gravel) and large (i.e. stones) sized substrates, suggesting coarser substrate patterns elicit a weaker pattern response than fine ones. When the fish were exposed to a background consisting of all three substrate sizes, the resulting pattern energy difference was similar to that elicited by the medium and large substrates.

It is well established that the size of the squares on a checkerboard affects the corresponding pattern elicited by both cuttlefish (Chiao & Hanlon, 2001; Barbosa *et al.*, 2007; Chiao, Chubb, & Hanlon, 2007) and some flatfish (Ramachandran *et al.*, 1996). We found no evidence that the check sizes used in this study had any effect on the type of pattern elicited by rock gobies, although further check sizes need testing. However, unlike cuttlefish, and perhaps some fishes such as slender filefish and certain flatfish, which may have a repertoire of different body patterns (Hanlon & Messenger, 1988; Kelman *et al.*, 2006; Allen *et al.*, 2015), it is likely that rock gobies have a limited repertoire of one or two patterns which they can express to varying degrees. This is similar to other pattern changing fish that appear to match their background using a limited repertoire of patterns (Lanzing, 1977; Watson, Siemann, & Hanlon, 2014; Allen *et al.*, 2015; Tyrie *et al.*, 2015), though there can still be considerable variation between and within individuals (Healey, 1999). For instance, the tropical flatfish *Bothus ocellatus* is thought to have three basic patterns (Ramachandran *et al.*, 1996), southern flounder (*Paralichthys lethostigma*) and winter flounder (*Pseudopleuronectes americanus*) have at least one pattern, and plaice (*Pleuronecte platessa*) have two (Kelman *et al.*, 2006). In addition Nassau groupers (*Epinephelus striatus*) have also

been shown to change between three basic body patterns within a few seconds (Watson *et al.*, 2014).

Our observations made during the experiments and in the field suggest that rock gobies may control the expression of one of two different pattern types. The two pattern types, here referred to as 'barred' and 'black square', are shown in Figure 3. It is not known whether a given individual is able to elicit both pattern types, as this was not observed in our study. It is, however, unlikely that these two pattern types are mutually exclusive and there are many similarities between them (e.g. Figures 3D and 3E). It should also be noted that the 'barred' pattern type was not observed in fish greater than 60 mm in length. The 'black square' pattern type was observed in fish of all sizes, but was most vivid and contrasting in larger individuals. While it is possible that these two pattern types result from sexual dimorphism, this has not been reported in any of the studies which investigated the life history of this species (Miller, 1961; Dunne, 1978; Azevedo & Simas, 2000; Hajji, 2012). While these markings could play a role in some form of signalling, the fact that the fish changed their pattern in response to different backgrounds suggests that they are, at least in part, important for camouflage. Furthermore, because fresh sea water was used for each fish, and there was no movement of water between the different sections of the tray, any pattern change in response to potential chemical cues from conspecifics should have been eliminated.

Due to the nature of rocky shores as heterogeneous environments, closely matching the background with fixed patterns is challenging and depends on the composition of the habitat patch. Instead, the patterning of rock gobies may have evolved as a compromise in camouflage on multiple backgrounds rather than to specialize on one background type (Merilaita, Tuomi, & Jormalainen, 1999; Houston,

Stevens, & Cuthill, 2007). Compared to the background matching abilities of other animals capable of rapid pattern change, almost all of the individuals tested in this study showed only a limited improvement in background matching, despite showing a large change in body pattern on all backgrounds. This suggests that background matching might not be the primary camouflage type used by rock gobies. For instance, both the 'barred' and 'black square' pattern types cross over the edge of the body, which is characteristic of disruptive coloration (Cott, 1940; Cuthill *et al.*, 2005; Stevens & Merilaita, 2009b).

Disruptive coloration has been defined as markings that hinder the detection or recognition of an animal's body outline by creating the illusion of false edges (Stevens & Merilaita, 2009b). Such markings have been shown to be particularly effective when they touch the edge of the animal's body because they break up the real body edges while also blending the animal's outline with the background (Cuthill *et al.*, 2005; Stevens & Cuthill, 2006; Stevens & Merilaita, 2009b). Fast visual detection of animals in natural scenes has been shown to depend heavily on information regarding visual edge and body outline, while chromatic information is less important (Delorme, Richard, & Fabre-Thorpe, 2000; Fei-Fei *et al.*, 2005; Stevens & Cuthill, 2006; Elder & Velisavljević, 2009). It is therefore plausible that the 'barred' and 'black square' patterns elicited by rock gobies are a form of disruptive coloration and thus help to conceal the animal by breaking up the outline of the body. Furthermore, disruptive camouflage can be an effective anti-predator strategy even if the overall combination of markings do not match the background entirely (Stevens & Cuthill, 2006; Schaefer & Stobbe, 2006), meaning that the markings could camouflage the fish even if background matching was poor (as was the case on the backgrounds tested in this study). This could

perhaps be tested using a model of edge detection to look at how much of the true body edges are intact under different scenarios (Stevens & Cuthill, 2006; Lovell *et al.*, 2013). To date, the majority of studies into disruptive coloration have been conducted in terrestrial environments and so future work should aim to investigate its function and effectiveness within marine environments.

The artificial backgrounds used in this study were designed to resemble the shape and size of natural backgrounds, (i.e. each of the four backgrounds could resemble a different rock pool area). However, they differed from natural backgrounds in that they did not contain any chromatic or three-dimensional information. Therefore, a potential limitation of this study is that we cannot be sure that the fish would respond in the same way to real substrates. For instance, plaice have been shown to change pattern almost instantly when moved from fine to coarse gravel of the same hue, but respond very differently when moved between artificial backgrounds (Healey, 1999). Cuttlefish have been found to show a stronger pattern change on real gravel than a 2D image of the gravel, though there was no difference between cuttlefish which were directly on the gravel and those viewing it through Perspex, indicating that they are indeed using visual cues (Kelman *et al.*, 2008; Allen *et al.*, 2009). None of the backgrounds used in this study elicited the full expression of the 'black square' in any individuals. The full expression of the 'black square' was only seen while observing the fish within rock pools (Figure 3B), suggesting that cues not present in the experimental backgrounds are also important. Future work should therefore investigate the conditions under which these different patterns are seen in the field.

This study has shown that rock gobies are capable of rapidly changing their body pattern in response to changes in their visual background. The ability to change

pattern for camouflage, whether it by via background matching, disruptive coloration, or a combination of several camouflage types, is likely to provide a survival advantage for animals occupying heterogeneous habitats such as the intertidal zone. It is therefore plausible that the ability to change body pattern for camouflage could be widespread not only among intertidal fish, but also among species that occupy other heterogeneous environments such as coral reefs (Marshall & Johnsen, 2011; Watson *et al.*, 2014). As has been noted previously, more comparative analyses are needed to understand the drivers of colour change for camouflage in different animal species (Stuart-Fox & Moussalli, 2009; Umbers *et al.*, 2014; Stevens, 2016).

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744

LIST OF FIGURES

768 the change in body pattern relative to the other backgrounds and time point. **(B)** PED
769 between the granularity spectra of the fish and that of the background there were placed
770 on at 0 and 15 min. The lower the PED the greater the level of background matching
771 relative to the other backgrounds and time point. Both graphs show medians plus inter-
772 quartile range (IQR), whiskers are lowest and highest values that are within 1.5*IQR
773 from the upper and lower quartiles, outliers are shown by dots.

774

775 **Figure 3:** The two basic pattern types, here referred to as 'barred' (left) and 'black
776 square' (right), identified in rock gobies on Gyllyngvase beach, Falmouth. **(A)** Barred
777 pattern fully expressed while observing the fish in a rock pool, **(B)** black square pattern
778 fully expressed while observing the fish in a rock pool, **(C)** barred pattern fully
779 expressed, **(D)** black square pattern partially expressed, **(E)** barred pattern partially
780 expressed, **(F)** barred pattern not expressed, and **(G)** black square pattern not expressed.